CYSTICERCOSIS/TAENIASIS: RECENT ADVANCES IN SEROLOGICAL AND MOLECULAR DIAGNOSES

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Abstract. Serodiagnosis by immunoblot, using recombinant chimeric *T. solium* antigen and native glycoprotein antigens, has been applied for neurocysticercosis cases. Specific antibodies against both antigens were detected in serum samples from NCC patients involving multiple cysts in the brain, whereas it was not always easy to detect specific antibodies in NCC cases with a solitary cyst or calcified lesion(s). On the other hand, the diagnosis for human taeniasis or worm carriers has been routinely performed by stool examination. In this study, multiplex PCR has been established to differentiate taeniasis using *Taenia* mitochondrial DNA in fecal samples from worm carriers. Furthermore, the molecular identification of human taeniid cestodes by base excision sequence scanning thymine-base analysis has also been introduced. This method provides four thymine-base peak profiles unique for Asian and American/African genotypes of *T. solium*, *T. saginata* and *T. asiatica*. By comparing thymine base peak profiles, it is possible to differentiate human taeniid cestodes without DNA sequencing. The approaches are powerful tools for the routine diagnosis of taeniasis and the molecular identification of taeniid cestodes.

INTRODUCTION

Neurocysticercosis (NCC), caused by the accidental ingestion of eggs of the pork tapeworm, *Taenia solium,* is one of the most serious zoonoses. The diagnosis of NCC has been routinely performed by image diagnosis and serodiagnosis. The diagnostic reliability of serodiagnosis has been drastically improved since purified glycoprotein and recombinant antigens have been developed. In contrast, taeniasis is caused by the ingestion of *T. solium, T. asiatica* or *T. saginata* metacestodes in undercooked pork or beef. However, its pathogenecity is not as severe as that of NCC. In addition, molecular approaches have been developed to differentiate human taeniid cestodes. In this review article, the most recent advances in 1) serodiagnosis for NCC using native glycoproteins and a recombinant chimeric antigen, 2) multiplex PCR for the detection of worm carriers with *T. solium* and *T. saginata*, and 3) base excision sequence scanning thymine-base (BESS T-base) analysis has been introduced for the precise molecular identification of human taeniid cestodes without DNA sequencing.

RECENT ADVANCES IN NCC DIAGNOSES

1) Serodiagnosis for neurocysticercosis using recombinant chimeric antigen

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In our department, glycoprotein antigens (GPs), purified by preparative isoelectric focusing from cyst fluid of *T. solium* metacestodes, have been routinely used for serodiagnosis for NCC (Ito *et al*, 1998; 2000). Recently, recombinant *T. solium* antigens have also been developed (Sako *et al*, 2000). In this article, immunoblot using recombinant chimeric *T. solium* antigen with a molecular mass of 17kDa has been introduced. The antigen genes were originally cloned from the cDNA library of the Asian genotype of *T. solium* metacestodes, and the two clones obtained, Ag1V1 and Ag2 encoding 7- and 10-kDa polypeptides, respectively, were fused by PCR mutagenesis to produce a chimeric antigen (Sako *et al*, 2000; 2001). The recombinant antigen did not show cross-reaction with serum samples from patients with alveolar and cystic echinococcosis, clonorchiasis, fascioliasis, sparganosis, schistosomiasis or paragonimiasis, indicating that the Ag1V1/Ag2 chimeric antigen is highly specific and useful as a differential serodiagnosis for NCC (Sako *et al*, 2000; 2001). The specificity of the chimeric antigen is higher than native GPs, but the sensitivity seems to be lower than native GPs (90% compared with 100% in native GPs). In this study, a total of 19 suspected and confirmed NCC cases, which we have experienced since 1996 in our department, were reexamined by immunoblots using native GPs and recombinant antigens. Seven of 11 NCC cases with multiple cystic lesions in the brain were serologically positive, whereas one of 8 NCC cases with a solitary or calcified lesion(s) was positive. This might be due to the different antibody responses stimulated by a solitary cyst, or multiple cysts. Most

recently, it has been demonstrated that native GPs and recombinant antigen are also useful for the diagnosis of cysticercosis in intermediate hosts, such as pigs and dogs (Sato *et al*, 2003; Ito *et al*, 2002a). In any event, at present, it would be recommended that ELISA and immunoblot using native GPs and/or recombinant chimeric antigens for the serodiagnosis of NCC be performed.

2) Molecular diagnosis of taeniasis by multiplex PCR

For the diagnosis of taeniasis with *T. solium* or *T. saginata*, stool examination is routinely performed. However, it is impossible to differentiate taeniid eggs based on morphology. Furthermore, the accurate identification even of proglottids or metacestodes may be subject to confusion. Several molecular approaches for the differential diagnosis of human taeniid cestodes have been developed (Flisser *et al*, 1988; Rishi and McManus, 1988; Harrison *et al*, 1990; Zarlenga *et al*, 1991; Bowles and McManus, 1994; Chapman *et al*, 1995). These approaches have provided sensitive and useful methods for the identification of taeniid cestodes. However, some are relatively time-consuming. Multiplex PCR, as reported by González *et al* (2000), is also sensitive for the differential diagnosis of taeniasis and echinococcosis, but genomic DNA extracted from tapeworms was used as template DNA. In this article, multiplex PCR using DNA samples extracted from feces of worm carriers has been established to differentiate worm carriers with *T. saginata* and *T. solium*. Copro-DNA was extracted from 0.2 g of fecal samples from worm carriers using QIAamp DNA Stool Mini kit (Qiagen). Mitochondrial DNA (mtDNA) prepared from taeniid cestodes was also used for comparison. The forward primers used for the amplification of cytochrome *C* oxidase subunit 1 gene (*cox 1*) were as follows: 5´-TTGATTCCTTCGATGGCTTTTCTTTTG-3´, 5´-ACGGTTGGATTAGATGTTAAGACTA-3´, 5´-GTTAGGAGGTGGTGATCCTGTTTTG-3´ and 5´-GGTAGATTTTTTAATGTTTTCTTTA-3´ for *T. saginata*, *T. asiatica,* Asian and American/African genotypes of *T. solium,* respectively. Reverse primer, 5´-GACATAACATAATGAAAATG-3´, was common to *cox 1* from human taeniid cestodes. The PCR protocol consisted of 30 cycles of denaturation (30 seconds at 94˚C), annealing (30 seconds at 60˚C), and extension (90 seconds at 72˚C plus one cycle of 4 minutes at 72˚C). In multiplex PCR using mtDNA, *cox 1* fragments with molecular sizes of 270, 827 and 483bp were amplified in *T. saginata*, *T. asiatica*, and the Asian genotype of *T. solium*, respectively (Fig 1-A, lanes 1-5). The *cox 1* fragments were detectable if at least 5 eggs were contained in 1 g of fecal sample (data not shown). Therefore, when multiplex PCR, using copro-DNA, was performed, diagnostic *cox 1* fragments with 827 and 483bp in sizes were also amplified in worm carriers with *T. saginata* (lanes 1-3 in Fig 1B) and Asian genotype of *T. solium* (lanes 4-6 in Fig 1B), respectively, although the diagnostic band was not so intense (lane 2 in Fig 1B). These results indicate that multiplex PCR is a highly sensitive and simple method for the differential diagnosis of human taeniasis. This method can also be applied for differential identification if parasite materials are available.

3) Molecular identification of human taeniid cestodes by BESS T-base analysis

As mentioned above, it is not always possible to precisely identify human taeniid cestodes based on morphology, in particular, in Asian regions where *T. saginata, T. asiatica* and *T. solium* are distributed sympatrically. In addition, most recently, it has been reported that two genotypes exist in *T. solium*, based on phylogenetic analysis using mitochondrial DNA (Okamoto *et al*, 2001; Ito *et al*, 2002b; Nakao *et al*, 2002). It is also impossible to differentiate these genotypes morphologically. In order to differentiate these taeniid cestodes, molecular approaches, such as PCR-restriction fragment length polymorphism (Bowles and McManus, 1994; Gasser and Chilton, 1995; Rodriguez-Hidalgo *et al*, 2002), single-strand conformation polymorphism (Gasser *et al*, 1999), and multiplex PCR (González *et al*, 2000) have been developed. Therefore, for the differential diagnosis of all human taeniid species, including two genotypes of *T. solium*, the base excision sequence scanning thymine-base (BESS T-base) method has been developed, using mitochondrial *cox 1* and cytochrome *b* gene (Yamasaki *et al*, 2002a, b). This method is for the detection only of thymine ("T") bases and the identification of taeniid cestodes by comparing diagnostic "T" base peak profiles. Based on the nucleotide sequences of *cox 1* from human taeniid cestodes, most "T" bases are well-conserved among taeniid species, but species- or genotype-specific "T" bases are sparsely distributed over the gene. Fig 2 shows the representative BESS T-base profiles unique for *T. saginata, T. asiatica* and two genotypes of *T. solium cox 1*. "T" bases at positions 189, 595, 603, 612 and 618 of the *cox 1* serve as differential markers and it has been confirmed that bases at these sites are completely conserved among species. For example, in *T. saginata,* "T" base peaks appear at positions 189 and 612, but no "T" base peaks appear at positions 595, 603 and 618, because bases at these positions are

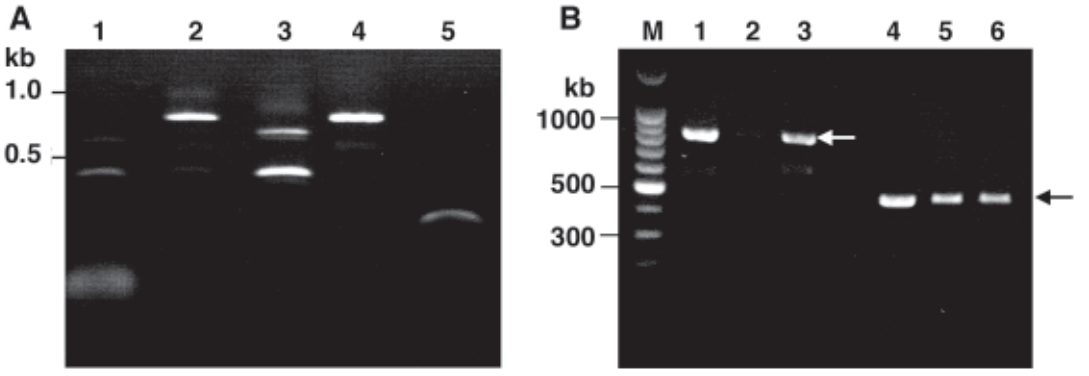

Fig 1- Multiplex PCR using cytochrome *c* oxidase subunit 1 gene fragments.
Panel A. Template DNAs were prepared from Asian genotype of *T. solium* (lanes 1 and 3), *T. saginata* (lanes 2 and 4), and *T. asiatica* (lane 5). The molecular sizes of diagnostic products are 827, 483 and 270bp for *T. saginata*, Asian genotype of *T. solium* and *T. asiatica*, respectively. Panel B. DNA samples extracted from fecal samples of worm carriers were used as template DNA. Arrows indicate diagnostic markers of 827bp for *T. saginata* worm carriers (lanes 1-3) and 483bp for *T. solium* Asian genotype infections (lanes 4-6), respectively, are shown.

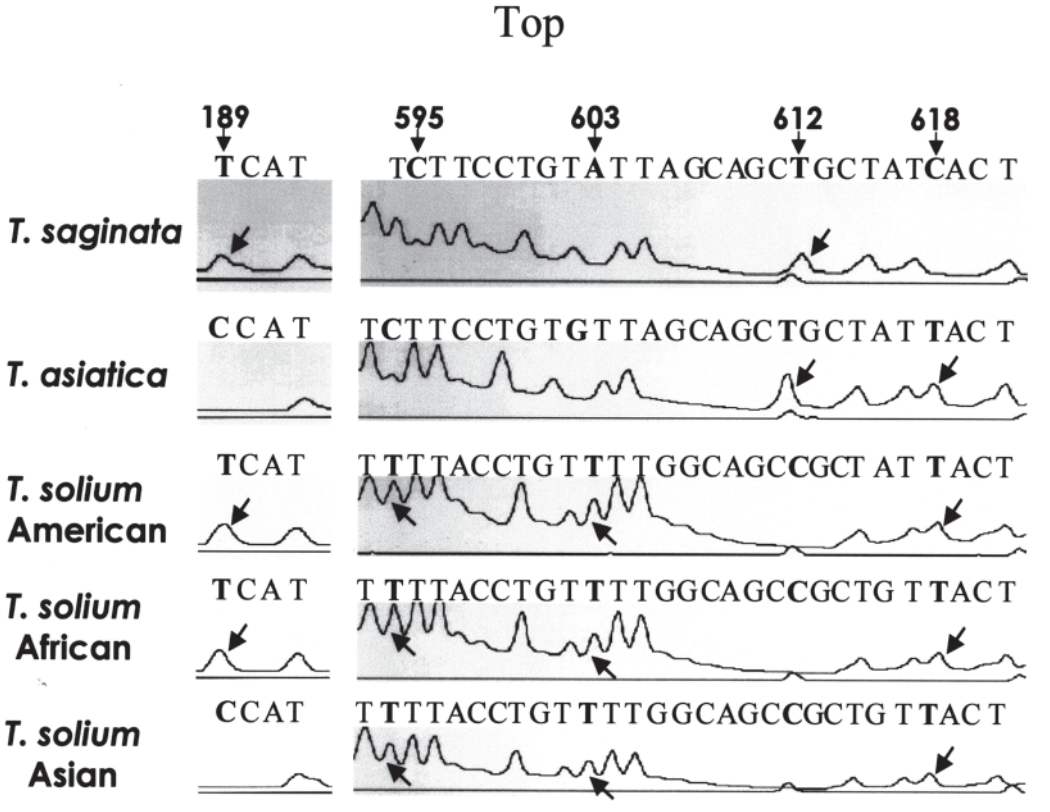

Fig 2- BESS T-base analysis using cytochrome *c* oxidase subunit 1 gene fragments. The representative BESS T-base profiles of *T. saginata* (China), *T. asiatica* (Taiwan), American/African and Asian genotypes of *T. solium* (Brazil, Tanzania and China) are shown. The nucleotide sequences indicated are from the databases (accession numbers, AB066495, AB066494, AB066492, AB066493 and AB066485). The diagnostic "T" bases are indicated with arrows.

not "T" base. If the parasite is *T. asiatica*, diagnostic " T" base peaks would appear at positions 612 and 618, but not 189, 595 and 603. When "T" base peaks appear at positions 595, 603 and 618 and disappear at position 612, the cestode can be *T. solium*, and the differential marker for the two genotypes of *T. solium* is the "T" base peak at position 189. In other words, if an additional "T" base peak appears at the position, the parasite has to be the American/African type of *T. solium*, whereas no "T" base peak appears at the position in the Asian genotype of *T. solium*. Thus, by comparing characteristic "T" base peak profiles, it is possible to differentiate human taeniid cestodes accurately and easily, without DNA sequencing. The BESS T-base system provides a powerful tool not only for the routine identification of human taeniid cestodes but also for the identification of a variety of organisms if nucleotide data are available.

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